# Is there a world beyond bevacizumab in targeting angiogenesis in glioblastoma?

Seystahl, K ; Weller, M

Abstract: INTRODUCTION: Antiangiogenic approaches are currently the dominating experimental therapeutic strategy in glioblastoma. First enthusiasm was provoked by promising radiological response rates and an apparent clinical benefit with some of these agents. Major limitations include the modest number of durable responses, the lack of cytotoxic antitumor activity, of synergy when combined with chemotherapy and of an overall survival benefit. AREAS COVERED: We review the rationale as well as preclinical and clinical evidence for the future development of antiangiogenic agents in glioblastoma. The most prominent approach targets VEGF and includes agents such as the VEGF antibody bevacizumab, the VEGF receptor fusion protein aflibercept or the tyrosine kinase inhibitors cediranib and XL-184. Inhibition of angiogenic pathways by small molecules, for example, enzastaurin, or anti-integrin-based approaches, for example, cilengitide, represent alternative strategies. EXPERT OPINION: Enzastaurin and cediranib failed in randomized Phase III trials in recurrent glioblastoma, aflibercept in Phase II. By contrast, bevacizumab was conditionally approved in many countries. Recently completed Phase III trials for bevacizumab and cilengitide in the first-line setting will define the future role of these agents. This intense clinical trial activity reflects the hope that antiangiogenic agents will become part of the limited therapeutic options for glioblastoma.

DOI: https://doi.org/10.1517/13543784.2012.670219

**Is there a world beyond bevacizumab in targeting angiogenesis in glioblastoma?**

## Abstract

***Introduction:*** Antiangiogenic approaches are currently the dominating experimental therapeutic strategy in glioblastoma. First enthusiasm was provoked by promising radiological response rates and an apparent clinical benefit with some of these agents. Major limitations include the modest number of durable responses, the lack of cytotoxic antitumor activity, of synergy when combined with chemotherapy and of an overall survival benefit.

***Areas covered:*** We review the rationale as well as preclinical and clinical evidence for the future development of antiangiogenic agents in glioblastoma. The most prominent approach targets vascular endothelial growth factor (VEGF) and includes agents such as the VEGF antibody bevacizumab, the VEGF receptor fusion protein aflibercept, or the tyrosine kinase inhibitors cediranib and XL-184. Inhibition of angiogenic pathways by small molecules, e.g. enzastaurin, or anti-integrin based approaches, e.g. cilengitide, represent alternative strategies.

***Expert opinion:*** Enzastaurin and cediranib failed in randomized phase III trials in recurrent glioblastoma, aflibercept in phase II. In contrast, bevacizumab was conditionally approved in many countries. Recently completed phase III trials for bevacizumab and cilengitide in the first-line setting will define the future role of these agents. This intense clinical trial activity reflects the hope that antiangiogenic agents will become part of the limited therapeutic options for glioblastoma.

Keywords: Aflibercept, Angiogenesis, Bevacizumab, Cediranib, Cilengitide, Clinical trials, Enzastaurin, Glioma, VEGF, XL-184

## 1. Introduction

Glioblastoma is the most common and most malignant primary brain tumor in adults. Despite advances in the understanding of genetic and biologic characteristics of this tumor, the prognosis remains poor with a median survival of approximately 10 months in recent population-based studies [1]. Since 2005, the standard of care of newly diagnosed glioblastoma includes surgery or biopsy followed by radiotherapy and concomitant and adjuvant temozolomide [2]. At tumor recurrence, there is no generally accepted salvage therapy. Competing regimens include temozolomide rechallenge commonly using a dose-intensified regimen [3], nitrosoureas [4, 5] or novel antiangiogenic agents. Since glioblastomas are highly vascularized tumors, various agents targeting angiogenesis have been tested in clinical trials. Here, we review the preclinical and clinical data on currently explored antiangiogenic agents and approaches.

### 1.1 Rationales for the use of antiangiogenic agents in glioma

The formation and development of blood vessels represent pivotal steps during embryogenesis. In the adult, physiological angiogenesis is restricted to the maintenance of tissue homeostasis and integrity which is required during wound healing, inflammation and the menstrual cycle [6]. The pioneers of angiogenesis research suggested that a tumor mass cannot exceed a diameter of 0.4 to 1.0 mm without establishing its own vasculature and recruiting a vascular network for further tumor growth [7]. Conceptually, the *de novo* formation of blood vessels was defined as vasculogenesis which is mediated by the differentiation of precursor cells into endothelial cells whereas angiogenesis represents the development of new vessels from a pre-existing vascular network [8]. In the last decades several milestones have been reached in the understanding of molecular mechanisms involved in this process.

### 1.2 Identification of key molecules in the process of angiogenesis

Basic and acidic fibroblast growth factor (bFGF/aFGF) which are secreted by various cancer cells were among the first signaling molecules shown to induce angiogenesis by stimulating the proliferation and differentiation of endothelial cells [9]. The 22 members of the FGF family, ranging from 17 to 34 kDa in size and interacting with tyrosine kinase FGF receptors, play an important role in multiple physiologic processes of embryonic development and wound healing, but can also promote pathologic angiogenesis [10].

With vascular endothelial growth factor (VEGF), a very potent and more specific angiogenic factor was identified, giving further support to the concept that tumors depend on the development of new blood vessels. VEGF-A is the predominant and most angiogenic of the vascular growth factors and exists in different isoforms derived from alternative splicing of an eight exon-bearing pre-mRNA [11]. Beside the proangiogenic isoforms VEGFxxx-a, "xxx" indicating the number of aminoacids (VEGF165a, VEGF189a, VEGF206a), also antiangiogenic isoforms containing a different c-terminal and named VEGFxxx-b have been described [11, 12].

VEGF-A stimulates proangiogenic signaling via binding to VEGF receptor (VEGFR)-2. The activity of VEGFR-2 can be modulated by the co-receptors and VEGF-binding neuropilins (NRP)-1 and NRP-2. VEGF is also a ligand of VEGFR-1 which may act as an antagonist of angiogenic signaling by trapping VEGF but exhibits simultanously only minor angiogenic signaling due to its weak tyrosine kinase activity [13]. The soluble variant of VEGFR-1 (sVEGFR-1), a truncated about 110 kDa splice variant of the 180 kDa transmembrane receptor VEGFR-1, was also been shown to trap VEGF [14]. VEGFR-1- and sVEGFR-1-related pathways might represent an endogenous control mechanism balancing pro- and antiangiogenic signaling in physiologic conditions and potentially aberrant proangiogenic

signaling in pathologic conditions like cancer. Of note, hypoxia which represents a major stimulatory factor of proangiogenic signaling, downregulates the expression of sVEGFR-1 in endothelial cells [15]. Some authors suggest that VEGF-VEGFR-2 signaling may represent an autocrine regulation pathway of glioma cells in addition to the paracrine interplay of tumor and endothelial cells [16, 17]. Further members of the VEGF family represent VEGF-B and placental growth factor (PlGF) which also bind VEGFR-1 and may act as competitive antagonists of VEGF-A. Furthermore PlGF has indirect effects on angiogenesis by stimulating different cell types to upregulate the production of VEGF-A and other angiogenic factors, e.g. FGFs, platelet-derived growth factor (PDGF), stromal cell-derived factor 1 (SDF1), granulocyte colony-stimulating factor (G-CSF) and matrix metalloproteinase (MMP)-9 [18]. PlGF may play an VEGF-independent role in pathologic angiogenesis since PlGF inhibition was shown to inhibit tumor growth by targeting tumor vessels of VEGF(R)-inhibitor resistant tumors [19]. PlGF-related signaling might thus represent an escape pathway mediating resistance to anti-VEGF targeted therapies [18, 20]. However, a pivotal role of PlGF in angiogenesis has also been disputed. Carmeliet and collegues provided evidence for a proangiogenic role in various pathologic conditions, mostly cancer models. Bais and colleagues first refuted any significant effect on tumor angiogenesis in 15 models but later correlated efficacy of anti-PlGF treatment with VEGFR-1 expression in tumor cells [21-23].

Beside the VEGF family other pathways and angiogenic factors play a role in the complex process of angiogenesis. Among these, the family of integrins is involved in modulating a pro- or antiangiogenic microenvironment. Integrins are membrane-bound heterodimeric proteins that can be activated by recognizing ligands of the extracellular matrix (ECM), for example laminins or vitronectins. Ligated integrins are involved in the regulation of migration, invasion and survival of endothelial cells, a process that is especially important in

conditions of tumor angiogenesis [24]. MMPs interacting with integrin signaling contribute to this crosstalk between endothelial cells, integrins and the ECM by releasing pro- or antiangiogenic factors, i. e. VEGF of FGFs from the ECM via proteolytic cleavage [25].

After the establishment of vessels a state of quiescence is followed under physiologic conditions. However, this state must be able to switch to angiogenic conditions if required, for example, in case of wound healing. This system of vessel quiescence, stabilization and, if required, destabilization is regulated by the family of angiopoietins (ANG) and its receptors Tie-1 and Tie-2. In absence of Tie-1, Tie-2 can be activated by ANG-1 and ANG-2, leading to an unresponsiveness to angiogenic signals. In presence of Tie-1 a high ratio of ANG-2/ANG-1 results in proangiogenic signaling and *vice-versa* [26].

### 1.3 Cellular targets of antiangiogenesis

The first therapeutic concepts of antiangiogenesis have almost exclusively focused on host-derived endothelial cells. They have been considered to be a stable element of the tumor microenvironment and the risk of developing resistance to therapeutic approaches was of minor concern. However, the experience of antiangiogenic therapies in cancer in general and especially in glioblastoma tells that resistance occurs both in a constitutive and an acquired manner. Constitutive resistance which translates into a lack of response to antiangiogenic therapy may be due to multiple proangiogenic pathways acting not only on VEGF signaling, but also involving FGF, PlGF, ANG family members and integrins [13]. The mechanisms of acquired resistance are even less well understood. Different hypotheses have been developed regarding the relationship of endothelial and glioma cells which may at least in part explain the phenomenon of acquired resistance. In 2001, Kunkel and colleagues showed that inhibiting VEGF-driven angiogenesis resulted in a cooption of preexisting cerebral vessels by glioma cells [27]. Another interesting phenomenon, called vasculogenic mimicry, was described by El Hallani and colleagues: glioblastoma stem-like cells adapt endothelial cell-

like properties and exhibit comparable gene expression resulting in part in a transdifferentiation into vessel-like structures, e.g. the expression of collagen IV without expression of the pan-endothelial cell marker CD34 [28]. Thus angiogenesis and angiogenic tumor growth may be, at least in part, independent of the recruitment of endothelial cells and independent of endothelial cell-targeted therapy. An even more radical point of view represents the concept that endothelial cells forming tumor blood vessels can develop from tumor stem-like cells. Data supporting this hypothesis have been provided by several groups independently, showing that a subset of endothelial cells within the tumor vessels share genetic aberrations which are also found in glioma stem-like cells [29-31]. In addition, the tumor cells developed an endothelial phenotype when cultured under endothelial cell conditions and in a xenograft mouse model, tumor vessels were shown to be of human origin [30]. Of note, VEGF and VEGFR-2 seem to be involved in the transition of "tumor endothelial progenitor cells" into endothelium while the prior step of differentiation of stem-like $CD133^+$ cells is independent of VEGF [29]. Inhibiting VEGF might thus reduce the development of tumor-derived endothelial cells. In contrast, Soda and colleagues suggested that this transdifferentiation pathway is independent of VEGF *in vitro* and may be even stimulated by inhibiting VEGF *in vivo* [31]. Further investigation is required to get a better understanding of the complex interplay of glioma and endothelial cells in order to develop suitable strategies inhibiting different forms of angiogenesis.

### 1.4 Putative mechanisms of action of antiangiogenic therapy

The most intuitive goal of a therapeutic approach targeting tumor vessels is to deprive the tumors of blood supply in order to reduce their capability of growth. The underlying mechanism of action would be vascular regression. Another hypothesis represents the concept that antiangiogenic therapy leads to a normalization of a formerly chaotic network of vessels,

thus facilitating drug delivery and thereby potentially improving the response to other tumor-specific therapies [32, 33]. Vessel normalization is also likely to reduce an hypoxic tumor environment which is one of the key pathologic factors of glioblastoma and may contribute to radioresistance. Interestingly, hypoxia also facilitates the conditions for so called "glioma-initiating cells" to maintain their stem-like phenotype [34]. In the clinic, antiangiogenic therapy may induce both vascular normalization and regression effects [35].

### 1.5 Potential side effects and evasion of antiangiogenic therapy

In parallel with the advances in understanding the molecular mechanisms and therapeutic options of tumor angiogenesis serious concerns arose regarding potential side effects of antiangiogenic therapy. Kunkel and colleagues first described an evasive phenotype after anti-VEGFR-targeted therapy in a mouse glioma model which is characterized by an VEGF-independent growth and the cooption of preexisting cerebral vessels [27]. This was supported by several groups reporting an increased invasiveness of tumors after antiangiogenic treatment, both in mice and human glioma and also other cancer models [36, 37]. Whether this concept of an increased local invasiveness triggered by antiangiogenic agents is clinically relevant remains controversial [38, 39].

## 2. Candidate antiangiogenic agents for glioblastoma

Here, we provide an overview about candidate antiangiogenic agents currently tested in phase II and phase III trials of glioblastoma. Figure 1 illustrates the different mechanisms of action of antiangiogenic agents in the interplay of endothelial cells and glioma cells and table 1 gives a short characterization of the different agents including their status in current clinical trials.

### 2.1 Bevacizumab

*Preclinical data*

Bevacizumab, a humanized monoclonal antibody against VEGF (Avastin®, Genentech, South San Francisco, CA, and Roche, Basel, Switzerland) is currently the most prominent antiangiogenic agent in the field of glioblastoma. It exhibits six VEGF-targeting residues preventing VEGF from binding to its receptors [40]. First proof of concept regarding indirect anti-tumors effect by targeting VEGF with this antibody was provided in an orthotopic glioma mouse model without affecting glioma cell viability *in vitro* [41]. More recently, $CD133^+$ stem-like glioma cells were shown to secrete elevated levels of VEGF with a proangiogenic impact both on *in vitro* angiogenesis models and on *in vivo* glioma xenograft models that could be suppressed by bevacizumab [42].

*Clinical data*

The first larger clinical studies using bevacizumab in patients with malignant glioma were published in 2007 [43, 44]. Vredenburgh and colleagues reported two cohorts of glioblastoma patients, one cohort of 23 patients receiving bevacizumab at 10 mg/kg plus irinotecan every 2 weeks, the second cohort of 12 patients with bevacizumab at 15 mg/kg every 21 days and irinotecan on days 1, 8, 22, and 29 [43]. The dose of irinotecan was 340 $mg/m^2$ in patients taking enzyme-inducing antiepileptic drugs (EIAED) and 125 $mg/m^2$ in patients not taking EIAED. The encouraging rates for progression-free survival (PFS) of 46% and the 6-months overall survival rate of 77% paved the way for further bevacizumab-based regimens in clinical trials in glioblastoma [44]. In 2009, Friedman and colleagues published the BRAIN trial which later on served to facilitate the accelerated approval of bevacizumab. In this non-comparative phase II trial, 167 patients with recurrent glioblastoma were randomized to be treated with bevacizumab 10 mg/kg every 2 weeks with or without irinotecan [45]. Accordingly, strictly speaking, this study explored the addition of irinotecan to bevacizumab and not bevacizumab per se. Patients in both arms showed comparative rates for 6-months PFS (42.6% for bevacizumab alone versus 50.3% for the combination) and similar median

overall survival (9.2 months for bevacizumab alone versus 8.9 months for the combination). However, the safety profile of bevacizumab monotherapy was considerably better than in the combination arm (46.4% versus 65.8% grade ≥ 3 adverse events) [45]. In parallel, Kreisl and colleagues conducted a similar phase II trial treating patients with recurrent glioblastoma with 10 mg/kg bevacizumab every two weeks, with an option to add irinotecan at progression. 6-months PFS was 29% and 6-months overall survival rate 57%, with a median overall survival of 31 weeks [46]. No patient showed significant benefit from the addition of irinotecan at progression under bevacizumab. The safety of bevacizumab as a single-agent is generally considered good. The most frequent reported grade 3 and 4 adverse events in the cohorts with bevacizumab monotherapy were arterial or venous thromboembolism (6% and 12.5%), arterial hypertension (8% and 4%), wound healing (2.4% and 0%), bowel perforation (0 and 3%) and proteinuria (0 and 3%) [45, 46]. In contrast to initial expectations, bevacizumab as a single agent does not lead to a major risk of cerebral hemorrhage in glioma patients. Some authors report that the risk may be augmented by concurrent anticoagulation, while others did not observe an increase of bleeding events [47, 48]. The comparison regarding the general risk of cerebral hemorrhage in glioblastoma with or without bevacizumab and/or anticoagulation has not been assessed in a prospective study.

Based on the response rate and presumed clinical benefit reported in the two above-mentioned phase II studies [45, 46], bevacizumab was conditionally approved in 2009 by the US Food and Drug Administration and subsequently in many other countries for the treatment of recurrent glioblastoma. In contrast, the European Medicines Agency denied the registration of the drug since there was no inclusion of a bevacizumab-free control arm in any trial and therefore no proven effect on overall survival [49]. Further concerns arose regarding the interpretation of the response rates. The response assessment was mainly based on the Macdonald radiographic criteria [45, 46] which may be inappropriate to evaluate

antiangiogenic therapies since the reduction in contrast-enhancement may reflect a restoration of the blood-brain barrier only and not signify anti-tumor growth effects [50]. The results from the bevacizumab trials, amongst others, necessitated to include non-contrast-enhancing tumor and thus integration of changes in T2-weighted/FLAIR sequences in the response assessment in the new RANO criteria.

The above-mentioned observations of enhanced infiltration as an escape mechanism from antiangiogenic therapy provoked an ongoing discussion regarding the patterns of progression and failure after bevacizumab. Several authors reported an increased risk of diffuse invasiveness after treatment with bevacizumab in high grade glioma patients [39, 51-53]. In contrast, there has also been contradictory evidence which suggests no higher incidence of a gliomatosis-like phenotype after treatment with bevacizumab [38, 54].

The future impact of bevacizumab in the therapy of glioblastoma will depend on the results of two large phase III trials in newly diagnosed glioblastoma which have completed accrual and will provide prospective data comparing bevacizumab-treated and untreated cohorts. Both the registration trial AVAglio (NCT00943826) and the RTOG-0825 trial (NCT00884741) evaluate the addition of bevacizumab to standard temozolomide-based radiochemotherapy in a similar randomized double-blind protocol with reasonable endpoints of overall survival and progression-free survival [55]. In contrast to AVAglio, the RTOG trial did not include patients with a stereotactic biopsy and was conducted in the US where bevacizumab was freely available at recurrence. The implementation of quality of life assessment in these trials will also help to evaluate whether the expected steroid sparing effect translates into improved quality of life. A comparison of patients treated with temozolomide-based radiochemotherapy plus bevacizumab first-line to historical controls indicated a gain in PFS, but not in OS, that was explained by the availability of bevacizumab at recurrence in the US [56]. This may indicate that the RTOG 0825 may miss the OS endpoint, too. On the other hand, this may be

different with AVAglio which is conducted in many countries where bevacizumab is not readily available at recurrence. Of note, these considerations all suppose that bevacizumab given at recurrence has an impact on survival. Finally, the data sets will clarify whether bevacizumab alters patterns of relapse when given as first-line treatment.

Interestingly, in contrast to adult glioma patients, a phase II study in pediatric gliomas did not show any objective response which suggests different biologic and angiogenic profiles of pediatric tumors in this respect [57]. Of note, among adult glioma patients, clinical data indicated that bevacizumab shows a better efficacy in elderly patients whose gliomas may be more VEGF-dependent [58].

### 2.2 Aflibercept

*Preclinical data*

With the rationale to improve anti-VEGF-targeted therapies, the idea to prevent VEGF from interacting with its receptors by administering decoy soluble receptors led to the development of aflibercept (Sanofi-Aventis, Paris, France and Regeneron Pharmaceuticals, Tarrytown, NY). This drug, also called "VEGF trap", is a fusion protein exposing both the domain of VEGFR-1 and VEGFR-2, thereby "trapping" all isoforms of VEGF-A and PlGF [59]. Aflibercept showed efficacy in several preclinical models of solid tumors [60-62]. In an orthotopic mouse glioma model aflibercept showed a survival benefit both in initial and advanced phases of tumor development. However, this study also described the development of a VEGF trap-resistant phenotype characterized by progressive tumor growth and increased invasiveness [63].

*Clinical data*

Based on the encouraging preclinical data, a single-arm phase II study in recurrent malignant glioma was conducted by the North American Brain Tumor Consortium. 42 patients with

recurrent glioblastoma and 16 patients with recurrent anaplastic glioma were treated with 4 mg/kg every two weeks i. v. aflibercept. The results were disappointing in every aspect. The primary endpoint was not met: 6-months PFS was only 7.7% for glioblastoma and 25% for anaplastic glioma patients. In addition, the toxicity of aflibercept, including CNS ischemia and systemic hemorrhage, was considerable and 25% of the patients were removed from the study for toxicity [64]. After this failure in phase II, aflibercept is not expected to play any role in treatment of glioblastoma. However, this may not be generalized to all concepts of co-targeting VEGF and PlGF which currently are under investigation in clinical trials. Since inhibiting VEGF should at least resemble the clinical benefit of bevacizumab and aflibercept is supposed to target VEGF, general doubts arise about the efficacy of the compound.

### 2.3 Cediranib

*Preclinical data*

Cediranib (AZD2171, Recentin®, AstraZeneca, London, United Kingdom) is an oral ATP-competitive inhibitor of the tyrosine kinase activity of all VEGF receptors targeting also the PDGF and c-Kit receptors [65]. First proof-of-principle was provided both by targeting *in vivo* angiogenesis models via inhibiting endochondral ossification or corpora luteal development and by anti-tumor effects in different solid tumor mouse models [66]. In three orthotopic mouse glioma models, cediranib-treated mice exhibited a survival benefit which occurred despite continuous tumor growth and correlated with a potent reduction of tumor edema presumably by vascular normalization effects [67]. Thus, concerns may arise whether the postulated antiangiogenic properties of cediranib translate into reduced tumor growth or simply reflect a reduced vascular permeability.

*Clinical data*

First clinical experience with cediranib derived from a phase I study in advanced solid tumors describing an acceptable toxicity profile up to an oral daily dose of 45 mg [68]. A small phase II study with 16 patients with recurrent glioblastoma, conducted with 45 mg daily, showed a clinically relevant effect on tumor edema and MRI-validated potency to induce vascular normalization [69]. After completion of the trial, a total of 31 patients were analyzed for a primary endpoint of 6-months PFS showing encouraging results of 25.8% and a high radiographic response rate as assessed by Macdonald criteria of 56.7% of patients [70]. Based on these results, a randomized multicenter phase III trial was conducted to compare the efficacy of cediranib alone or in combination with lomustine in recurrent glioblastoma (REGAL; NCT00777153). 325 patients were randomized from 67 centers and ten countries on a 2:2:1 ratio, 131 patients receiving cediranib monotherapy (30 mg daily), 129 patients the combination of cediranib (20 mg daily) and lomustine (110 mg/m$^2$ every 6 weeks), and 65 patients receiving lomustine and an oral placebo as a control arm [71]. The PFS as primary endpoint was disappointing as there was no statistically significant difference of the cediranib-containing regimens (median PFS of 92 days for cediranib alone and 125 days in combination with lomustine) compared to the median PFS of 82 days of the control group. The 6-months PFS of 16% for cediranib monotherapy was considerably lower than the 25.8% of the phase II trial. This may be due to a lower dose of cediranib in the phase III trial or patient selection. However, the toxicity of 45 mg/day as administered in the phase II trial was considerable with 12.9% grade 3/4 toxicities, mainly hypertension, diarrhea and fatigue and high percentage (48.4%) of patients requiring drug interruption for toxicity. Nevertheless, another prospective randomized trial assessing the efficacy of cediranib has started enrollment, conducted by the Radiation Oncology Therapy Group (NCT01062425), to explore the addition of cediranib to standard temozolomide-based radiochemotherapy in newly diagnosed glioblastoma.

## 2.4 Cabozantinib (XL-184)

*Preclinical data*

Cabozantinib (XL184, Exelixis®, San Francisco, CA) is a dual tyrosine kinase inhibitor targeting the receptors MET and VEGFR-2. The MET ligand HGF also binds NRP-1 and thus may be related to VEGFR-2 proangiogenic signaling [72]. However, HGF-MET signaling is also involved in processes of migration and invasion in glioma and thus represents a promising therapeutic target [73]. In a mouse glioma model the proliferation of tumor and endothelial cells was reduced, resulting in a dose-dependent inhibition of tumor growth [74]. Interestingly, this effect was not associated with an increased metastatic spread in this model of lung cancer which has been observed in other approaches targeting VEGF-related pathways.

*Clinical data*

Cabozantinib is currently tested in a phase I dose finding study for newly diagnosed glioblastoma in combination with the standard therapy and has completed accrual for a phase II clinical trial in patients with progressive or recurrent glioblastoma (NCT00704288).

First results of an interim analysis were presented for 124 patients at ASCO 2010. The study was conducted in two cohorts, receiving 125 mg/day or 175 mg/day respectively. The cohort with 175 mg/day was reported to have a 6-months PFS of 21 % and an overall response rate of 21% in patients without and 8% in patients with prior antiangiogenic therapy. At that time, the 6-months PFS for the 125 mg cohort was not yet available. However, the overall response rate of the patients without prior antiangiogenic therapy was reported to be 32 %. Further evaluation of the risk and benefit is warranted after more follow-up data are available [75]. Regarding the toxicity profile the currently only available information from this trial is that

125 mg were better tolerated. A previous report of a phase II study in thyroid cancer reported a considerable toxicity profile including diarrhea, fatigue, palmar plantar erythrodysesthesia, nausea, thromboembolism, mucositis and elevation of liver enzymes [76].

### 2.5 Enzastaurin

*Preclinical data*

Enzastaurin HCl (LY317615, Eli Lilly and Company, Indianapolis, Indiana) is an acyclic bisindolylmaleimide, acting as a ATP-competitive inhibitor of protein kinase C-β (PKC-β) [77]. PKC-β is involved in the downstream signaling of VEGF [78]. The compound showed an inhibitory effect on plasma VEGF levels in small lung cancer and renal cell carcinoma mouse tumor models suggesting antiangiogenic activity [79]. In human glioblastoma orthotopic mouse models Enzastaurin suppressed tumor growth by inducing apoptosis in tumor cells and prolonged survival in synergy with irradiation [80, 81].

*Clinical data*

A phase I/II trial was conducted in recurrent glioma with an accrual of 118 patients [82]. In the phase I cohorts three dose levels (525 mg, 700 mg, 900 mg) were explored in patients receiving EIAED. Therapy was mostly well tolerated and the serum levels of patients on EIAED were approximately 80% lower than in those not on EIAED. The most common grade 3/4 adverse events included thrombosis, thrombocytopenia, hemorrhage and elevated alanine aminotransferase levels. In the phase II cohorts, patients not receiving EIAED were treated with 500 or 525 mg/day. The radiographic response rates of 84 evaluable patients were at 25%. Unfortunately this initial response did not translate into a considerable effect on disease control as the 6-months PFS was only 7% for patients with glioblastoma and 16% for patients with anaplastic glioma. Despite these modest results a phase III trial for patients with

recurrent glioblastoma was started comparing 500 mg enzastaurin/day with lomustine (100 to 130 mg/m$^2$ every 6 weeks) [5]. The enrolment was stopped after 266 of 397 initially planned patients since an interim analysis of PFS and OS did not show any significant difference between the treatment with lomustine. Median PFS for enzastaurin was at 1.5 months versus 1.6 months for lomustine and overall survival at 6.1 versus 7.1 months, respectively. In line with these disappointing results, a phase II study in patients with newly diagnosed glioblastoma exploring the addition of enzastaurin at 250 mg/day to standard temozolomide radiochemotherapy did not provide evidence for further benefit [83].

### 2.6 Cilengitide

*Preclinical data*

Cilengitide® (Merck KgaA, Darmstadt, Germany) is a synthetic Arginine-Glycine-Asparagine< (RGD) pentapeptide binding to the RGD ligand binding site on the integrin receptors αvβ3 and αvβ5 [84]. This interferes with the communication of integrins with their ligands in the extracellular matrix resulting in an impaired signaling to the cell. Inhibiting αvβ3 and αvβ5 integrins counteracts VEGF- or FGF-induced endothelial cell invasion and differentiation which suggests antiangiogenic activity for cilengitide [85]. Interestingly, tumor growth in orthotopic mouse models of glioblastoma and medulloblastoma was inhibited by cilengitide, but unaffected in case of subcutaneous implantation [86]. The effect of cilengitide thus may be highly context-dependent and cilengitide might modulate the tumor microenvironment towards a less permissive angiogenic phenotype. *In vitro*, cilengitide had no significant effect on tumor cell survival or proliferation and did not alter the sensitivity to temozolomide or irradiation [87]. However, *in vivo*, combining cilengitide with radiotherapy led to a survival benefit in an orthotopic mouse model compared with either modality alone [88].

*Clinical data*

The clinical experience in phase I clinical studies demonstrated a favorable safety profile without determination of maximum tolerated dose or dose limiting toxicity up to 2400 mg, administered intravenously twice weekly [89, 90]. A randomized phase II trial of 81 patients with recurrent glioblastoma comparing 500 mg versus 2000 mg cilengitide twice weekly as a monotherapy confirmed the excellent tolerability. A trend for a better activity was observed at the higher dose of 2000 mg cilengitide with a 6-months PFS of 15% and a median OS of 9.9 months [91]. However, the activity of cilengitide as a single agent was not considered to be sufficient and preclinical data suggested synergistic effects when combining it with irradiation [88]. The combination of cilengitide at 500 mg with standard radiation and temozolomide therapy in 52 patients with newly diagnosed glioblastoma was assessed in a phase II clinical trial. The results were encouraging with a 6-months PFS of 69%, a median overall survival of 16.1 months and 2-year survival rate of 35%. Striking was a post-hoc subgroup analysis of patients with a methylated $O^6$-methylguanine-DNA methyltransferase *(MGMT)* promotor. Preferentially these patients appeared to show the best benefit from the addition of cilengitide to the standard of care. The currently most favored hypothesis for this benefit represents the idea of a vascular normalization effect leading to an improved delivery of temozolomide to the tumor cells. Another phase II trial of cilengitide in newly diagnosed glioblastoma randomized patients to 500 mg versus 2000 mg cilengitide in addition to standard radiochemotherapy. Data of an interim analysis were presented at ASCO 2009, indicating an estimated median survival time of 18.9 months and overall survival at 12 months for all patients of 79.5% [92]. The design of subsequent phase III trials has been based on the observation of a pronounced benefit for patients bearing the *MGMT* promotor methylation [93]. As the first international multicenter clinical trial the CENTRIC trial incorporated the

screening for a molecular marker in the eligibility criteria [94]. Patients with verified methylated *MGMT* promotor were randomized either to the experimental arm receiving 2000 mg cilengitide in addition to standard radiochemotherapy or to the control arm receiving standard therapy alone. After completion of radiochemotherapy maintainance of cilengitide was intended to be given for up to 18 months. For patients with an unmethylated *MGMT* promotor a phase II trial, the CORE trial, compares the addition of 2000 mg cilengitide twice weekly (Arm 1) or five times a week (Arm 2) during radiotherapy, followed by cilengitide maintenance, to standard temozolomide radiochemotherapy alone (NCT00813943).

### 2.7 Other agents

A detailed characterization of all approaches targeting angiogenesis in glioblastoma would be beyond the scope of this review. Briefly, we here summarize some other concepts: Chemotherapy in a metronomic, i. e. continuous schedule, is postulated to have an antiangiogenic effect by targeting proliferating endothelial cells [95]. In recurrent glioblastoma, the activity reported for a temozolomide rechallenge with a continuous dose-intensified regimen might, at least in part, be explained by an effect on tumor angiogenesis [3]. There is also a broad range of small molecules aiming at inhibiting angiogenic pathways which were evaluated in phase I/II glioblastoma trials. Among these, the tyrosine kinase inhibitors sorafenib and sunitinib so far failed to show relevant activity in recurrent glioblastoma, but were associated with considerable toxicity [96, 97]. Temsirolimus (Torisel®, Wyeth Pharmaceuticals, Madison, NJ), currently under investigation in phase II glioblastoma trials (NCT01019434), is an inhibitor of the phosphoinositide 3-kinase/ mammalian target of rapamycin (mTOR) signaling pathway which can be overexpressed in glioblastomas and is involved in cell cycle regulation. Based on the experiences in renal cell carcinoma an antiangiogenic effect of the drug is postulated [98]. Vascular disruption is a

novel concept aiming at completely compromising the established structure of the tumor vasculature. In glioblastoma, first experience has been published for the α-tubulin antagonist CYT997 which showed vascular disrupting efficacy in preclinical models and in 3 of 6 evaluable patients in an ongoing phase I study in recurrent glioblastoma [99]. Cerebrovascular toxicity was within dose-limiting adverse events and will be of concern in the future development of this concept.

## 3. Conclusion

A growing understanding of key mechanisms of angiogenesis led to the development of various antiangiogenic agents with activity in preclinical glioma models. However, these findings did not always translate into progression or survival benefit in clinical trials. Several agents failed to prove efficacy in phase II and phase III trials. The monoclonal antibody against VEGF, bevacizumab, was approved as the first antiangiogenic drug in the treatment of (recurrent) glioblastoma mainly because of its activity on progression-free survival and a presumed clinical benefit. Currently conducted phase III trials for bevacizumab and cilengitide will define the future role of these two candidate antiangiogenic agents in glioblastoma.

## 4. Expert opinion

Which lessons have we learned from the clinical trials with antiangiogenic therapy in glioblastoma?

First, promising preclinical data often fail to translate into clinical benefit and especially survival benefit for patients. Sophisticated therapeutic approaches by small molecule targeting of tyrosine kinases and signaling molecules with predictable results *in vitro* may have pleiotropic effects with doubtful results in the complex system of tumor growth and

angiogenesis in the clinic. Predicting the mechanism of action and clinical outcome based on *in vitro* and even *in vivo* models seems more difficult than ever despite advances in our understanding of pivotal molecular mechanisms.

Second, there is more to consider than VEGF and VEGF inhibition in targeting angiogenesis in glioblastoma, but still the concepts have to be improved and also to be confirmed. A more profound understanding of the molecular mechanisms of angiogenesis led to the entry of small molecules like enzastaurin or cediranib into glioblastoma clinical trials, but also these agent failed in randomized phase III trials in recurrent glioblastoma. The future role of bevacizumab in glioblastoma will depend on the results of the currently conducted phase III trials. The conditional approval status of bevacizumab in recurrent glioblastoma in the US and many other countries throughout the world might be revised in case of failure in the first-line setting. The available phase II data for cilengitide indicate activity of the drug especially in patients with *MGMT* promotor methylation. First results of the phase III trial will be available in 2013.

Third, antiangiogenic agents alone are unlikely to be very effective. However, for bevacizumab, so far no clinical trial in glioblastoma showed superiority of a combined regimen compared to bevacizumab alone. The addition of cytotoxic agents e.g. irinotecan or carboplatin, but also small molecules like erlotinib did not show additional benefit but mostly was associated with an unfavorable safety profile [46, 100, 101]. The lack of efficacy may be due to the fact that some of the cytotoxic agents that have been applied simply do not have activity on glioma cells and thus an increased availability of the drug does not improve their efficacy. Second, the effect of vascular normalization may not only affect the intratumoral vessels but may also restore a formerly impaired blood-brain barrier which could lead to a reduced bioavailability of the drugs. However, the combination of different antiangiogenic

agents might be a promising approach based on the current understanding of VEGF-dependent and VEGF-independent angiogenic signaling. Despite the failure of aflibercept, co-targeting VEGF and PlGF remains a potential concept to overcome resistance to antiangiogenic therapies. A very promising combination partner of bevacizumab but also other antiangiogenic agents represents radiotherapy. Since vascular normalization may result in an improved oxygenation of the tissue, this is likely to increase the effect of ionizing radiation. Preclinical evidence for this concept comes from a mouse glioma model [102]. A recent phase II study of 25 patients with recurrent glioma treated with hypofractionated radiotherapy plus bevacizumab reported a 6-months PFS of 65%, suggesting activity of this concept [103].

Fourth, clinical trials should have a design which enables valid conclusions implicating reasonable endpoints, an appropriate response assessment tailored for the evaluation of antiangiogenic therapies and the use of verum-free control arms, that is without the investigative agent. [50]. The future design of clinical trials in glioblastoma will depend on the outcome of the two large randomized trials for primary diagnosed glioblastoma. The approval of either cilengitide or bevacizumab in the first-line setting would strongly affect the future design of clinical trials in glioblastoma.

In conclusion, the intense clinical trial activity reflects the current hope that antiangiogenic agents will become part of the still very limited therapeutic options for glioblastoma.

**Article highlights.**

- **Key pathologic features of glioblastoma represent a high vascularity and hypoxic tumor microenvironment. In addition, glioma cells express VEGF which plays a central role in tumor angiogenesis.**

- **Antiangiogenic treatment has entered the clinic in glioblastoma. The VEGF antibody bevacizumab is approved in many countries for the treatment of recurrent glioblastoma. The majority of current clinical trials focus on various antiangiogenic agents, both in newly diagnosed glioblastoma and in recurrent disease.**

- **Based on preclinical studies and radiographic assessments, one postulated mechanism of action of antiangiogenic treatment represents the vascular normalization of a formerly disorganized network of vessels. This could lead to a better drug delivery and better oxygenation and thereby potentially improved response to chemotherapy or irradiation**

- **Recent studies raised concerns regarding potential side-effects of antiangiogenic treatment in gliomas, especially the development of a proinvasive phenotype. A better understanding of the mechanism of action but also of failure of antiangiogenic treatment will help to overcome primary or acquired resistance. (Co-)Targeting other angiogenic factors beside VEGF or inhibiting pathways related to tumor invasion may represent reasonable strategies.**

- **There is evidence for activity of some antiangiogenic agents, especially bevacizumab and cilengitide, but other agents like aflibercept, cediranib and**

**enzastaurin failed in phase II or phase III trials. However, the effect of the currently available antiangiogenic agents may be restricted on improvement of progression-free survival and reduction of edema. Recently completed phase III trials for newly diagnosed glioblastoma will provide evidence whether bevacizumab or cilengitide have an effect on overall survival.**

**Abbreviations:**

ANG (Angiopoietin), ATP (Adenosine triphosphate), ECM (Extracellular matrix), EIAED (Enzyme-inducing antiepileptic drugs), FGF (Fibroblast growth factor), HGF (Hepatocyte growth factor), NRP (Neuropilin), OS (Overall survival), PDGFR (Platelet-derived growth factor receptor), PKC-β (Protein kinase C-β), PlGF (Placental growth factor), PFS (Progression-free survial), RGD (Arginine-Glycine-Asparagine), VEGF (Vascular endothelial growth factor), VEGFR (VEGF receptor)

**Figure 1:** Mode of action of antiangiogenic drugs: an overview

Figure legend:

Antiangiogenic drugs counteract angiogenic signaling at different extracellular and intracellular levels. The monoclonal antibody bevacizumab and receptor fusion protein aflibercept inhibit VEGF or VEGF plus PlGF, respectively, in the interaction with their receptors. Cilengitide interferes with the interaction of integrins with the ligands of the extracellular matrix. Cediranib and cabozantinib (XL-184) inhibit the tyrosine kinase activity of their corresponding receptors while enzastaurin targets downstream signaling by inhibiting protein-kinase-C-β.

Abbreviations: BEV (Bevacizumab), ECM (Extracellular matrix), HGF (Hepatocyte growth factor), NRP (Neuropilin), PKC-β (Protein kinase C-β), PlGF (Placental growth factor), VEGF (Vascular endothelial growth factor), VEGFR (VEGF receptor)

**References:**

1. Johnson DR, O'Neill BP. Glioblastoma survival in the United States before and during the temozolomide era. J Neurooncol. 2011; [Epub ahead of print]

2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. New England Journal of Medicine 2005; 352:987-96.

3. Perry JR, Belanger K, Mason WP, et al. Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. Journal of Clinical Oncology 2010; 28:2051-7.

4. van den Bent MJ, Brandes AA, Rampling R, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC Brain Tumor Group study 26034. Journal of Clinical Oncology 2009; 27:1268-74.

5. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. Journal of Clinical Oncology 2010; 28:1168-74.

6. Chung AS, Lee J, Ferrara N. Targeting the tumour vasculature: insights from physiological angiogenesis. Nat Rev Cancer 2010; 10:505-14.

7. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996; 86:353-64.

*** A historical, but still relevant review about the hypothesis of the angiogenic switch.**

8. Risau W, Flamme I. Vasculogenesis. Annual Review of Cell and Developmental Biology 1995; 11:73-91.

9. Klagsbrun M, Sasse J, Sullivan R, et al. Human tumor cells synthesize an endothelial cell growth factor that is structurally related to basic fibroblast growth factor. Proceedings of the National Academy of Sciences 1986; 83:2448-52.

10. Lieu C, Heymach J, Overman M, et al. Beyond VEGF: Inhibition of the fibroblast growth factor pathway and antiangiogenesis. Clinical Cancer Research 2011; 17:6130-9.

11. Robinson CJ, Stringer SE. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. Journal of Cell Science 2001; 114:853-65.

12. Bates DO, Cui T-G, Doughty JM, et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. Cancer Research 2002; 62:4123-31.

13. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473:298-307.

*** An excellent review about the current understanding of angiogenesis and translational research.**

14. Wu FTH, Stefanini MO, Gabhann FM, et al. A systems biology perspective on sVEGFR1: its biological function, pathogenic role and therapeutic use. Journal of Cellular and Molecular Medicine 2009; 14:528-52.

15. Ikeda T, Sun L, Tsuruoka N, et al. Hypoxia down-regulates sFlt-1 (sVEGFR-1) expression in human microvascular endothelial cells by a mechanism involving mRNA alternative processing. Biochemical Journal 2011; 436:399-407.

16. Knizetova P, Ehrmann J, Hlobilkova A, et al. Autocrine regulation of glioblastoma cell-cycle progression, viability and radioresistance through the VEGF-VEGFR2 (KDR) interplay. Cell Cycle 2008; 7:2553-61.

17. Steiner H-H, Karcher S, Mueller MM, et al. Autocrine pathways of the vascular endothelial growth factor (VEGF) in glioblastoma multiforme: clinical relevance of radiation-induced increase of VEGF levels. Journal of Neuro-Oncology 2004; 66:129-38.

18. Fischer C, Mazzone M, Jonckx B, et al. FLT1 and its ligands VEGFB and PlGF: drug targets for antiangiogenic therapy? Nat Rev Cancer 2008; 8:942-56.

19. Fischer C, Jonckx B, Mazzone M, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 2007; 131:463-75.

20. Bagley RG, Ren Y, Weber W, et al. Placental growth factor upregulation is a host response to antiangiogenic therapy. Clinical Cancer Research 2011; 17:976-88.

21. Bais C, Wu X, Yao J, et al. PlGF blockade does not inhibit angiogenesis during primary tumor growth. Cell 2010; 141:166-77.

22. Van de Veire S, Stalmans I, Heindryckx F, et al. Further pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. Cell 2010; 141:178-90.

23. Yao J, Wu X, Zhuang G, et al. Expression of a functional VEGFR-1 in tumor cells is a major determinant of anti-PlGF antibodies efficacy. Proceedings of the National Academy of Sciences 2011; 108:11590-5.

*** The identification of the expression of VEGFR-1 as potential factor for the efficacy of anti-PlGF antibodies might explain the conflicting findings regarding the role of PlGF inhibition in antiangiogenic treatment.**

24. Robinson SD, Hodivala-Dilke KM. The role of β3-integrins in tumor angiogenesis: context is everything. Current Opinion in Cell Biology 2011; 23:630-7.

25. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2:161-74.

26. Seegar TCM, Eller B, Tzvetkova-Robev D, et al. Tie1-Tie2 interactions mediate functional differences between angiopoietin ligands. Molecular Cell 2010; 37:643-55.

27. Kunkel P, Ulbricht U, Bohlen P, et al. Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor-2. Cancer Research 2001; 61:6624-8.

28. El Hallani S, Boisselier B, Peglion F, et al. A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. Brain 2010; 133:973-82.

**** Surprising insights into the biology of glioma cells suggesting the potential of a transdifferention of tumor cells into cells with endothelial properties.**

29. Wang R, Chadalavada K, Wilshire J, et al. Glioblastoma stem-like cells give rise to tumour endothelium. Nature 2010; 468:829-33.

30. Ricci-Vitiani L, Pallini R, Biffoni M, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature 2010; 468:824-8.

31. Soda Y, Marumoto T, Friedmann-Morvinski D, et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proceedings of the National Academy of Sciences 2011; 108:4274-80.

32. Ma J, Chen C-S, Blute T, et al. Antiangiogenesis enhances intratumoral drug retention. Cancer Research 2011; 71:2675-85.

33. Dickson PV, Hamner JB, Sims TL, et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clinical Cancer Research 2007; 13:3942-50.

34. Heddleston JM, Li Z, McLendon RE, et al. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle 2009; 8:3274-84.

35. Thompson EM, Frenkel EP, Neuwelt EA. The paradoxical effect of bevacizumab in the therapy of malignant gliomas. Neurology 2011; 76:87-93.

36. Pàez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 2009; 15:220-31.

*** A key report about the hypothesis that antiangiogenic therapy leads to a proinvasive phenotype of the tumor.**

37. de Groot JF, Fuller G, Kumar AJ, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro-Oncology 2010; 12:233-42.

38. Wick A, Dörner N, Schäfer N, et al. Bevacizumab does not increase the risk of remote relapse in malignant glioma. Annals of Neurology 2011; 69:586-92.

39. Narayana A, Kunnakkat SD, Medabalmi P, et al. Change in pattern of relapse after antiangiogenic therapy in high-grade glioma. Int J Radiat Oncol Biol Phys 2012;82:77-82.

40. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Research 1997; 57:4593-9.

41. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993; 362:841-4.

42. Bao S, Wu Q, Sathornsumetee S, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Research 2006; 66:7843-8.

43. Vredenburgh JJ, Desjardins A, Herndon JE, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. Journal of Clinical Oncology 2007; 25:4722-9.

44. Vredenburgh JJ, Desjardins A, Herndon JE, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clinical Cancer Research 2007; 13:1253-9.

45. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. Journal of Clinical Oncology 2009; 27:4733-40.

*** The approval of bevacizumab in recurrent glioblastoma is mainly based on this study and its data on response rates and progression-free survival.**

46. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. Journal of Clinical Oncology 2009; 27:740-5.

47. Norden A, Bartolomeo J, Tanaka S, et al. Safety of concurrent bevacizumab therapy and anticoagulation in glioma patients. Journal of Neuro-Oncology 2011:1-5.

48. Nghiemphu PL, Green RM, Pope WB, et al. Safety of anticoagulation use and bevacizumab in patients with glioma. Neuro Oncol 2008; 10:355-60.

49. Wick W, Weller M, van den Bent M, et al. Bevacizumab and recurrent malignant gliomas: a european perspective. Journal of Clinical Oncology 2010; 28:e188-e9.

50. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of Clinical Oncology 2010; 28:1963-72.

*** The RANO criteria represent an approach to identify appropriate response criteria for the evaluation of antiangiogenic therapy.**

51. Verhoeff J, van Tellingen O, Claes A, et al. Concerns about antiangiogenic treatment in patients with glioblastoma multiforme. BMC Cancer 2009; 9:444.

52. de Groot JF, Fuller G, Kumar AJ, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro-Oncology 2010; 12:233-42.

53. Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas. Neurology 2008; 70:779-87.

54. Pope WB, Xia Q, Paton VE, et al. Patterns of progression in patients with recurrent glioblastoma treated with bevacizumab. Neurology 2011; 76:432-7.

55. Chinot O, de La Motte Rouge T, Moore N, et al. AVAglio: Phase 3 trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme. Advances in Therapy 2011; 28:334-40.

56. Lai A, Tran A, Nghiemphu PL, et al. Phase II Study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. Journal of Clinical Oncology 2011; 29:142-8.

57. Gururangan S, Chi SN, Young Poussaint T et al. Lack of efficacy of bevacizumab plus irinotecan in children with recurrent malignant glioma and diffuse brainstem glioma: a Pediatric Brain Tumor Consortium study. Journal of Clinical Oncology 2010; 28:3069-75

58. Nghiemphu PL, Liu W, Lee Y et al. Bevacizumab and chemotherapy for recurrent glioblastoma: a single-institution experience. Neurology 2009; 72:1217-22.

59. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: A VEGF blocker with potent antitumor effects. Proceedings of the National Academy of Sciences 2002; 99:11393-8.

60. Byrne AT, Ross L, Holash J, et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. Clinical Cancer Research 2003; 9:5721-8.

61. Kim ES, Serur A, Huang J, et al. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. Proceedings of the National Academy of Sciences 2002; 99:11399-404.

62. Verheul HMW, Hammers H, van Erp K, et al. Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. Clinical Cancer Research 2007; 13:4201-8.

63. Gomez-Manzano C, Holash J, Fueyo J, et al. VEGF Trap induces antiglioma effect at different stages of disease. Neuro-Oncology 2008; 10:940-5.

64. de Groot JF, Lamborn KR, Chang SM, et al. Phase II study of aflibercept in recurrent malignant glioma: a North American Brain Tumor Consortium study. Journal of Clinical Oncology 2011; 29:2689-95.

*** Negative phase II trial of aflibercept in recurrent glioma.**

65. Brave SR, Ratcliffe K, Wilson Z, et al. Assessing the activity of cediranib, a VEGFR-2/3 tyrosine kinase inhibitor, against VEGFR-1 and members of the structurally related PDGFR family. Molecular Cancer Therapeutics 2011; 10:861-73.

66. Wedge SR, Kendrew J, Hennequin LF, et al. AZD2171: A highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Research 2005; 65:4389-400.

67. Kamoun WS, Ley CD, Farrar CT, et al. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. Journal of Clinical Oncology 2009; 27:2542-52.

*** In this preclinical study the survival benefit of mice treated with cediranib was due to a reduction of tumor edema despite continuous tumor growth.**

68. Drevs J, Siegert P, Medinger M, et al. Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. Journal of Clinical Oncology 2007; 25:3045-54.

69. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007; 11:83-95.

70. Batchelor TT, Duda DG, di Tomaso E, et al. Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. Journal of Clinical Oncology 2010; 28:2817-23.

71. Batchelor TT, Mulholland P, Neyns B, et al. The efficacy of cediranib as monotherapy and in combination with lomustine compared to lomustine alone in patients with recurrent glioblastoma: a phase III randomized study. Neuro Oncol 2010; 12: suppl 4, iv69-iv78, abstract OT-25.

*** Report about the failure of cediranib in phase III.**

72. Hu B, Guo P, Bar-Joseph I, et al. Neuropilin-1 promotes human glioma progression through potentiating the activity of the HGF//SF autocrine pathway. Oncogene 2007; 26:5577-86.

73. Wick W, Grimmel C, Wild-Bode C, et al. Ezrin-dependent promotion of glioma cell clonogenicity, motility, and invasion mediated by BCL-2 and transforming growth factor-β2. The Journal of Neuroscience 2001; 21:3360-8.

74. Yakes FM, Chen J, Tan J, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Molecular Cancer Therapeutics 2011; 10: 2298-308.

75. Wen PY, Prados M, Schiff D, et al. Phase II study of XL184 (BMS 907351), an inhibitor of MET, VEGFR2, and RET, in patients with progressive glioblastoma. J Clin Oncol 2010; 28: suppl; abstract 2006.

76. Kurzrock R, Sherman SI, Ball DW, et al. Activity of XL184 (Cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. Journal of Clinical Oncology 2011; 29:2660-6.

77. Faul MM, Gillig JR, Jirousek MR, et al. Acyclic N-(azacycloalkyl)bisindolylmaleimides: isozyme selective inhibitors of PKCβ. Bioorg Med Chem Lett 2003; 13:1857-9.

78. Suzuma K, Takahara N, Suzuma I, et al. Characterization of protein kinase C β isoform's action on retinoblastoma protein phosphorylation, vascular endothelial growth factor-induced endothelial cell proliferation, and retinal neovascularization. Proceedings of the National Academy of Sciences 2002; 99:721-6.

79. Keyes K, Mann L, Sherman M, et al. LY317615 decreases plasma VEGF levels in human tumor xenograft-bearing mice. Cancer Chemotherapy and Pharmacology 2004; 53:133-40.

80. Graff JR, McNulty AM, Hanna KR, et al. The protein kinase Cβ-selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. Cancer Research 2005; 65:7462-9.

81. Tabatabai G, Frank B, Wick A, et al. Synergistic antiglioma activity of radiotherapy and enzastaurin. Annals of Neurology 2007; 61:153-61.

82. Kreisl TN, Kotliarova S, Butman JA, et al. A phase I/II trial of enzastaurin in patients with recurrent high-grade gliomas. Neuro-Oncology 2010; 12:181-9.

83. Butowski N, Chang SM, Lamborn KR, et al. Phase II and pharmacogenomics study of enzastaurin plus temozolomide during and following radiation therapy in patients with newly diagnosed glioblastoma multiforme and gliosarcoma. Neuro-Oncology 2011; 13:1331-8.

84. Goodman SL, Hölzemann G, Sulyok AG, et al. Nanomolar small molecule inhibitors for alphav(beta)5, and alphav(beta)3 integrins. Journal of Medicinal Chemistry 2002; 45:1045-51.

85. Nisato R, Tille J-C, Jonczyk A, et al. αv β 3 and αv β 5 integrin antagonists inhibit angiogenesis in vitro. Angiogenesis 2003; 6:105-19.

86. MacDonald TJ, Taga T, Shimada H, et al. Preferential susceptibility of brain tumors to the antiangiogenic effects of an alpha(v) integrin antagonist. Neurosurgery 2001; 48:151-7.

87. Maurer GD, Tritschler I, Adams B, et al. Cilengitide modulates attachment and viability of human glioma cells, but not sensitivity to irradiation or temozolomide in vitro. Neuro-Oncology 2009; 11:747-56.

88. Mikkelsen T, Brodie C, Finniss S, et al. Radiation sensitization of glioblastoma by cilengitide has unanticipated schedule-dependency. International Journal of Cancer 2009; 124:2719-27.

89. MacDonald TJ, Stewart CF, Kocak M, et al. Phase I clinical trial of cilengitide in children with refractory brain tumors: Pediatric Brain Tumor Consortium Study PBTC-012. Journal of Clinical Oncology 2008; 26:919-24.

90. Nabors LB, Mikkelsen T, Rosenfeld SS, et al. Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. Journal of Clinical Oncology 2007; 25:1651-7.

91. Reardon DA, Fink KL, Mikkelsen T, et al. Randomized Phase II Study of Cilengitide, an Integrin-Targeting Arginine-Glycine-Aspartic Acid Peptide, in Recurrent Glioblastoma Multiforme. Journal of Clinical Oncology 2008; 26:5610-7.

92. Nabors LB, Mikkelsen T, Batchelor T, et al. NABTT 0306: A randomized phase II trial of EMD 121974 in conjunction with concomitant and adjuvant temozolomide with radiation therapy in patients with newly diagnosed glioblastoma multiforme (GBM). J Clin Oncol 2009; 27:suppl; abstract 2001)

93. Stupp R, Hegi ME, Neyns B, et al. Phase I/IIa Study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. Journal of Clinical Oncology 2010; 28:2712-8.

**** The first clinical trial in glioblastoma incorporating the screening for a molecular marker in the eligibility criteria.**

94. Stupp R, Bent MJVD, Erridge SC, et al. Cilengitide in newly diagnosed glioblastoma with MGMT promoter methylation: protocol of a multicenter, randomized, open-label, controlled phase III trial (CENTRIC). J Clin Oncol 2010; 28: suppl; abstract TPS152.

95. Kerbel RS, Kamen BA. The antiangiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004; 4:423-36.

96. Neyns B, Sadones J, Chaskis C, et al. Phase II study of sunitinib malate in patients with recurrent high-grade glioma. Journal of Neuro-Oncology 2011; 103:491-501.

97. Wen PY, Cloughesy T, Kuhn J, et al. Phase I/II study of sorafenib and temsirolimus for patients with recurrent glioblastoma (GBM) (NABTC 05-02). J Clin Oncol 2009; 27: suppl; abstract 2006.

98. Le Tourneau C, Faivre S, Serova M, et al. mTORC1 inhibitors: is temsirolimus in renal cancer telling us how they really work? Br J Cancer 2008; 99:1197-203.

99. Lickliter J, Fida R, Wheeler H, et al. Carboplatin combined with the vascular-disrupting agent CYT997 for recurrent glioblastoma multiforme. J Clin Oncol 2010; 28: suppl; abstract e13591)

100. Reardon D, Desjardins A, Peters K, et al. Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naïve, recurrent glioblastoma. Journal of Neuro-Oncology 2011; [Epub ahead of print]

101. Sathornsumetee S, Desjardins A, Vredenburgh JJ, et al. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. Neuro-Oncology 2010; 12:1300-10.

102. McGee MC, Hamner JB, Williams RF, et al. Improved intratumoral oxygenation through vascular normalization increases glioma sensitivity to ionizing radiation. Int J Radiat Oncol Biol Phys 2010; 76: 1537-45.

103. Gutin PH, Iwamoto FM, Beal K et al. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. Int J Radiat Oncol Biol Phys. 2009; 75:156-63

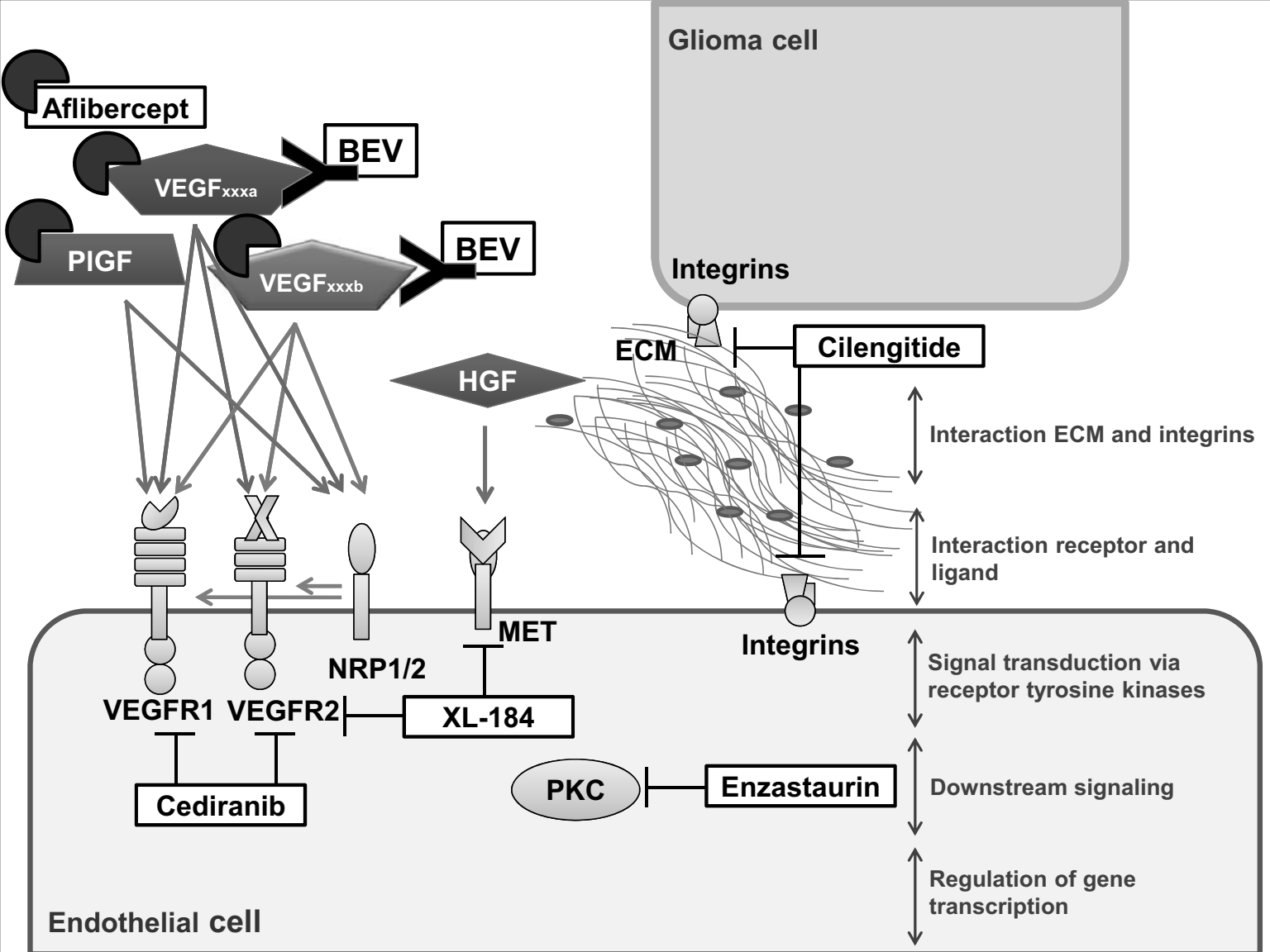
Glioma cell
Integrins
Aflibercept
BEV
VEGFxxxa
PIGF
BEV
VEGFxxxb
ECM
Cilengitide
HGF
Interaction ECM and integrins
Interaction receptor and ligand
MET
Integrins
NRP1/2
VEGFR1
VEGFR2
XL-184
Signal transduction via receptor tyrosine kinases
PKC
Enzastaurin
Downstream signaling
Cediranib
Regulation of gene transcription
Endothelial cell

**Table 1:** Salient features of antiangiogenic agents evaluated in clinical trials in glioblastoma

| Agent | Mode of action | Clinical status | Administration | Safety profile | Ref. |
|---|---|---|---|---|---|
| **Bevacizumab** | Monoclonal antibody against human VEGF preventing the interaction of VEGF with its receptors | Approved for recurrent glioblastoma in various countries,<br>Phase III for newly diagnosed glioblastoma (AVAglio, RTOG0825) | 10 mg/kg every 2 weeks i.v. | Favorable:<br>arterial hypertension, thromboembolism, impaired wound healing | [45, 46] |
| **Aflibercept** | IgG fusion protein, exhibiting domains of VEGFR-1 and VEGFR-2, thereby binding VEGF-A and PlGF | Phase II in recurrent glioblastoma negative | 4 mg/kg every 2 weeks i.v. | Considerable:<br>CNS ischemias, systemic hemorrhage, fatigue, thromboembolism, impaired wound healing | [64] |
| **Cediranib** | Tyrosine kinase inhibitor targeting kinases of VEGFR-1, -2, -3 and PDGFR and c-Kit impairing their signal transduction | Phase III in recurrent glioblastoma negative (REGAL) | 45 mg/day p.o. (phase II),<br>30 mg/day p.o. (phase III monotherapy)<br>20 mg/day p.o. (phase III combination with lomustine) | Considerable:<br>hypertension, diarrhea, fatigue, elevation of liver enzymes | [70, 71] |
| **Cabozantinib** | Dual tyrosine | Phase I/II | 125 mg or 175 | Safety data for glioma not yet | [75, |

| | | | | | |
|---|---|---|---|---|---|
| | kinase inhibitor targeting kinases of MET and VEGFR-2 impairing their signal transduction | | mg/day p.o. | available; data from a phase II trial in thyroid cancer:<br><br>diarrhea, fatigue, palmar plantar erythrodysesthesia, nausea, thromboembolism, mucositis, elevation of liver enzymes | 76] |
| **Enzastaurin** | ATP-competitive inhibitor of protein kinase C-β, inhibition of downstream signaling of VEGF and other pathways | Phase III in recurrent glioblastoma negative (STEERING) | 525 mg/500 mg (phase II)<br><br>250 mg (phase III) | Good:<br><br>thromboembolism, thrombocytopenia, hemorrhage, elevation of liver enzymes | [5, 82] |
| **Cilengitide** | Pentapeptide, inhibitor of integrins αvβ3 and αvβ5 by binding to their RGD binding site | Phase II in recurrent glioblastoma,<br><br>phase III in newly diagnosed glioblastoma (CENTRIC) | 500 mg or 2000 mg | Excellent:<br><br>no specific toxicities known | [89, 90] |

Abbreviations: ATP (Adenosine triphosphate), PlGF (Placental growth factor), PDGFR (Platelet-derived growth factor receptor), RGD (Arginine-Glycine-Asparagine), VEGF (Vascular endothelial growth factor), VEGFR (VEGF receptor)